REMARKS/ARGUMENTS

Reconsideration of this application and entry of the foregoing amendments are respectfully requested.

Claim 1 has been amended to specify that the inducer of Hepatic Stellate cell (HSC) apoptosis employed is sulfasalazine or a derivative of sulfasalazine capable of inducing HSC apoptosis. That amendment finds basis in previous claim 23. Claim 1 has also been amended to specify that the liver disease being treated is liver fibrosis. That amendment finds basis at the paragraph bridging pages 6 and 7 of the application as filed. New claim 30 has been added which refers to a number of specific sulfasalazine derivatives and finds basis at page 16, lines 18 to 22 of the application as filed. Claims 3-8, 15, 16, 23 and 29 have been cancelled without prejudice.

The claims as now presented are drawn to the subject matter of Group VII in the Restriction Requirement dated October 13, 2005 and hence relate to the use of sulfasalazine and derivatives thereof capable of inducing HSC apoptosis in treating liver fibrosis. Claims relating to the other inventions identified in the Restriction Requirement have been withdrawn from consideration. Applicant reserves the right to file a continuation application to one or more of those inventions and indeed any subject matter withdrawn from consideration or cancelled here.

Claims 1-8, 11, 15, 16 and 22-29 stand rejected under 35 USC 112, first paragraph, as allegedly lacking written description. Claims 1-8, 11, 15, 16 and 23-27 stand rejected under 35 USC 112, first paragraph, as allegedly being non-enabled. Withdrawal of these rejections is submitted to be in order in view of the claim amendments and for the reasons that follow.

In order to facilitate prosecution, the claims have been amended and now specify that:

- the condition to be treated is liver fibrosis; and

- the inducer of Hepatic Stellate Cell (HSC) apoptosis employed is sulfasalazine or a derivative thereof capable of inducing HSC apoptosis.

It is respectfully submitted that adequate written description is provided for the claims as presented and that the subject matter of the claims is enabled.

As acknowledged in the Official Action, the Examples of the present application provide experimental data demonstrating that:

- sulfasalazine is able to induce apoptosis of HSCs in culture; and
- sulfasalazine is able to bring about resolution of liver fibrosis in a rat model of the condition induced by treatment with carbon tertrachloride.

Thus, the present application provides actual *in vivo* experimental evidence showing that liver fibrosis can be resolved in a mammalian model.

Animal models are used as a reliable way of assessing therapies. The model of liver fibrosis employed in the present application is a model that is recognized and used as a close model of human liver fibrosis. Given that the application demonstrates successful treatment of liver fibrosis in that model, it is entirely reasonable that the claims cover the use of sulfasalazine or derivatives thereof capable of inducing apoptosis in HSCs to treat liver fibrosis.

Liver fibrosis embraces a group of liver diseases that have a common underlying mechanism of the buildup of abnormal connective tissue in the liver as a result of liver damage. Hepatic Stellate Cells play a role in the build-up and development of liver fibrosis, secreting various extracellular matrix proteins and enzymes. The description of the present application describes a number of conditions involving liver fibrosis over pages 6 to 9. As all of these conditions involve activated HSCs and liver damage giving rise to the fibrosis, the breadth of the claims is reasonable in covering liver fibrosis in general, because of the common underlying pathology.

The Official Action also focuses on the issues of selectivity and means of delivery. The present application describes a number of ways that inducers of HSC application can be delivered providing adequate written description. In particular, the description describes administration routes and delivery methods over pages 10 to 21, including methods ranging from injection, liposomes, implants and viral delivery.

Importantly though, sulfasalazine and its derivatives are selective inducers of HSC apoptosis because they induce apoptosis of HSCs, but do not induce apoptosis of other liver cells to any extent. That means selective delivery of sulfasalazine is not essential.

For instance, Example 5 of the present application demonstrates that sulfasalazine is selective by showing that liver fibrosis is resolved in rats in which fibrosis had been induced by carbon tetrachloride. Examination of the livers in Example 5 following treatment showed reduction in the number of HSCs and resolution of fibrosis, it did not show apoptosis of other cells and damage to the liver that might be expected if sulfasalazine was not selective.

Example 5 therefore demonstrates that sulfasalazine can be successfully employed to treat liver fibrosis without needing selective delivery. Thus, the application does provide adequate written description for the claims and the treatment of liver fibrosis. The application shows that the inventors were in full possession of the invention.

A copy of the literature paper, Oakley *et al* (2005) Gastroenterology, 128: 108-120 is provided which provides further confirmatory evidence of the ability of sulfasalazine and its derivatives to treat liver fibrosis and of their selectivity. In Oakley *et al* the administration of sulfasalazine is demonstrated to only result in the significant apoptosis of HSCs and not of other cell types in the liver.

Oakley *et al* indicates at page 112, right hand column that a 64% drop in α-SMA positive HSCs is seen, whereas there was no significant drop in macrophages and states at the paragraph bridging page 113 that:

> *"No appreciable differences were observed in total TUNEL positive cells per field between the sulfasalazine-treated and untreated livers, thus indicating that sulfasalazine is unlikely to significantly influence hepatocyte apoptosis"*

Oakley goes on to indicate that study of liver enzyme function also shows that sulfasalazine has no effect on hepatocyte viability. Thus, Oakley provides further confirmation of the experimental data provided in the present application showing that sulfasalazine only induces apoptosis of HSCs and does not have a significant effect on the other cells in the liver. Selective delivery is not therefore essential, because sulfasalazine itself is selective.

The Official Action also cites some literature references and argues that they would have meant the skilled person would have considered that uncertainty surrounded the use of sulfasalazine and its derivatives. However, that is not the case and nothing in the references detracts from the demonstration that liver fibrosis can be treated using sulfasalazine in the present application. In particular, considering each reference in turn:

(i) Bataller *et al* (2005)

The Official Action cites Bataller *et al* as indicating that some therapies may not be actively taken up by activated HSCs and hence may result in unwanted side-effects due to their effect on other cells. As discussed above, Example 5 of the present application demonstrates that sulfasalazine itself selectively induces apoptosis of HSCs and not of other cells in the liver and hence can be used successfully.

The Official Action also cites Bataller *et al* in relation to the use of animal models. As discussed above, and acknowledged in Bataller *et al*, animal models are widely used.

Furthermore, Bataller *et al* indicates liver biopsy in clinical trials in humans is an invasive technique that can cause pain and major complications (see page 210, left hand column final line to right hand column line 2). Thus, animal models do represent the widely used and accepted approach because of the difficulties in carrying out human trials.

Indeed, for a publication that the Official Action argues questions the validity of animal studies, Bataller *et al* quotes widely from them (see page 209, left hand column, final five lines, page 231, right hand column, final paragraph to page 214, left hand column, line 19, page 214, right hand column, final paragraph and also Table 2). It appears Bataller *et al*, like those in the field, does rely on, and acknowledge the validity of, animal studies.

The fact that the carbon tetrachloride model of liver fibrosis employed in the Examples of the present application was one widely employed at the time of the invention, and would be a model considered to be highly credible by the skilled person, can be further demonstrated by performing a simple Medline search.

By performing a Medline search using the terms "carbon tetrachloride" and "liver fibrosis" one obtains over 1000 results for the period from 1970 to the priority date of the present application. A copy of the summary of the results of the Medline search, the limits set and of the first twenty results is attached. A review of the first 20 results shows that carbon tetrachloride model was widely used. Thus, it is evident that the skilled person would have considered the results of the carbon tertrachloride model credible and that it was reasonable to extrapolate them to the treatment of liver fibrosis in humans. People in the art did rely on the model.

Thus, the use of the rat model of liver fibrosis in the present application is a valid one and does represent adequate written description.

(ii) Watkinson *et al* (1986) and Marinos *et al* (1992)

Neither of Watkinson *et al* and Marinos *et al* would stop the skilled person from administering sulfasalazine and its derivatives. That is simply not the case and the skilled person at the time of the invention would consider sulfasalazine a safe, well-tested drug that had been administered for a number of conditions for a long period of time.

The abstract of Watkinson *et al* indicates that:

> "*Sulfasalazine, devised by Dr Nana Svartz for the treatment of 'infective polyarthritis' has been used in the treatment of inflammatory bowel disease for more than 40 years*" [emphasis added]

The abstract goes on to conclude that:

> "*Side effects are common but are mainly reversible and not serious..... Sulfasalazine remains a most useful drug in the treatment of inflammatory bowel disease after 40 years of use*" [emphasis added]

A document that states that sulfasalazine remains an important drug for use in the treatment of inflammatory bowel disease is not something that gives any indication that the skilled person would be deterred from using sulfasalazine and its derivatives. Indeed, the contrary appears true.

Marinos *et al* refers to two case studies where patients who had been administered sulfasalazine subsequently died. Marinos *et al* acknowledges that it is possible that sulfasalazine was not responsible for either mortality stating at page 134, right hand column that:

> "*it is possible that both these patients had fulminant hepatic failure of unknown etiology...*"

Indeed, as indicated at page 133, right hand column, lines 12 and 13, the first patient died due to a fungal infection following liver transplant. Thus, the link to sulfasalazine is tenuous at best. Furthermore, as Marinos *et al* indicates that any link was due to hypersensitivity to the treatment (see page 135, left hand column, second full paragraph), patients can be monitored for

adverse reactions such as rashes, fever and other symptoms to sulfasalazine and administration stopped.

Nothing in Marinos *et al* would prevent the skilled person considering employing sulfasalazine and its derivatives. Sulfasalazine had been administered to hundreds of thousands of people and as acknowledged in Marinos *et al* only four mortalities had previously been seen in subjects (see page 132, right hand column, first paragraph).

Drugs such as penicillin and aspirin are routinely given even though a minority of people are severely allergic to them. Similarly the miniscule number of severe adverse reactions would not prevent the skilled person from using sulfasalazine and its derivatives in the treatment of liver fibrosis.

Thus, nothing in Watkinson *et al* and Marinos *et al* would have deterred the skilled person from considering administering sulfasalazine and its derivatives to treat liver fibrosis. The fact that sulfasalazine is still routinely prescribed for a number of conditions, such as arthritis, shows that Watkinson *et al* and Marinos *et al* have not changed the fact that sulfasalazine is seen as a safe and effective drug.

Indeed, sulfasalazine is presently licensed in the UK for the treatment of Inflammatory Bowel Disease (IBD) and colitis. The fact that regulatory bodies have approved the use of sulfasalazine and continue to do so, shows that sulfasalazine is regarded as a safe and effective drug that can be administered to patients.

Thus, in summary the claimed invention is adequately described and it is evident the inventors were in full possession of the invention at the priority date. The application provides experimental evidence showing that sulfasalazine can be used to treat liver fibrosis in an accepted model of the condition and that it selectively induces the apoptosis of HSCs to achieve

that effect. The description also provides adequate detail on how to put the invention into practice.

Reconsideration is requested.

Claims 1-8, 11, 15, 16 and 23-29 stand rejected under 35 USC 103 as allegedly being obvious over Liptay *et al* and Lang *et al*. The rejection is traversed.

Lang *et al* is concerned with gene regulation in the Hepatic Stellate Cell and looks at the expression of a wide variety of genes in HSCs. Lang *et al* also notes that HSCs in the diseased liver undergo apoptosis. Liptay *et al* indicates that Sulfasalazine may be used to induce apoptosis in T lymphocytes in culture. However, nothing in the two documents, either alone or in combination with each other, would have led the skilled person to employ sulfasalazine to treat liver fibrosis as now specified by claim 1 for the reasons set out below.

In particular, the skilled person would have considered that artificially inducing apoptosis could have actually had a detrimental effect, rather than a therapeutic effect and would not, in any case, have considered that an inducer of T cell apoptosis would be effective for inducing HSC apoptosis and therefore treating liver fibrosis. The present inventors were the first to demonstrate *in vivo* that sulfasalazine could be successfully employed.

(i) *The skilled person would have had no reasonable expectation of success that the artificial induction of Hepatic Stellate Cell apoptosis would be a way to treat liver fibrosis*

Lang *et al* would not have provided the skilled person with any reasonable expectation of success that they would be able to artificially induce HSC apoptosis to treat liver fibrosis.

Lang *et al* is concerned with the study of events in liver fibrosis and the role of HSCs in the diseased liver. At page 177, right hand column, final full paragraph, Lang *et al* indicates that

Retinoic acid can be used to reverse the activation of HSCs and speculates that such an effect could be important in the natural resolution of liver fibrosis and mentions that an alternative mechanism would be the induction of apoptosis of HSCs. Starting from Lang *et al* the skilled person could well have chosen the option of attempting to reverse activation of HSCs, rather than seeking to induce their apoptosis.

Furthermore, even if the skilled person had considered artificially inducing apoptosis, they would not have any reasonable expectation of success. Importantly, Lang *et al* provides no experimental data on the artificial induction of HSC apoptosis *in vivo*. Without such *in vivo* data, the skilled person would have considered that inducing HSC apoptosis was just as likely to have a detrimental effect, rather than be a way to treat liver fibrosis.

In particular, as outlined at page 6, lines 11 to 24 of the present application:

- the natural development and resolution of liver fibrosis is complex with a large number of factors playing a role including HSCs, the skilled person would have considered that the artificial induction of apoptosis, using an inducer not corresponding to the natural stimulus, could well result in a profound disturbance of liver structure and function due to the elimination of HSCs, rather than the resolution of liver fibrosis; and

- the skilled person would have considered that the natural clearance mechanisms for the removal of apoptotic cells could be overloaded by the artificial induction of apoptosis, meaning that apoptotic cells would not be removed and could become necrotic damaging the liver, not promoting the resolution of liver fibrosis.

Thus, prior to the present invention, the skilled person would have had no reasonable expectation of success that the artificial induction of HSC apoptosis would be a way to successfully treat liver fibrosis. The present inventors were the first to demonstrate that it could be successfully done. The subject matter of the claims is inventive for that reason.

(ii) *Even if the skilled person had considered artificially inducing HSC apoptosis, they would not have considered employing sulfasalazine to do so*

Even if the skilled person had considered artificially inducing HSC apoptosis, they would not have considered employing sulfasalazine.

Firstly, Liptay *et al* is in a different technical field to the present invention. It is not concerned with treating liver disease, rather it is concerned with T cells and Chronic Inflammatory Bowel Disease (IBD). Secondly, and most importantly, Liptay *et al* is concerned with T cells and provides only *in vitro* results with the T cell line RBL5. There is no indication in Liptay *et al* that sulfasalazine would have any effect on HSCs, indeed HSCs are not even mentioned.

Sulfasalazine does not induce the apoptosis of all cell types, indeed sulfasalazine is highly selective and only induces the apoptosis of a narrow range of cells. There is therefore no reason why the skilled person would have considered that sulfasalazine would be able to induce HSC apoptosis, particularly given that Liptay *et al* makes no mention of HSCs.

The Examiner has the full benefit of hindsight, but looking at the documents at the time of the invention, the skilled person is given no reason to consider HSCs from Liptay *et al* or that sulfasalazine would effect HSCs in the same way as the RBL5 T cell line. The skilled person would not have considered that results from one cell line could simply be extrapolated to another and there are numerous examples were that is not the case.

The Official Action focuses on reference in Liptay *et al* to NFκB inhibition and inducing apoptosis in T lymphocytes and also to reference to NFκB in Lang *et al*. However, the skilled person would have been well aware that the role of NFκB in apoptosis is complicated and would not have extrapolated results seen in T lymphocytes to HSCs.

A copy of Lin *et al* (1995) *The Journal of Cell Biology*, 131(5):1149-1161 is provided. Lin *et al* is concerned with the induction of apoptosis in a prostate carcinoma cell line and a

neuroblastoma cell line by Sindbis virus (SV) and the effect of oxidative stress. Lin *et al* indicates at page 1150, right hand column, first full paragraph that:

> *"These results define NF-kappa B activation as a necessary component of SV induced apoptosis in AT-3 cells and suggest that thiol agents such as Bcl-2 may inhibit apoptosis, in part, by acting on an NF-kappa B signalling pathway."* [emphasis added]

Thus, whereas Liptay *et al* indicates that inhibiting NFκB can lead to T cell apoptosis, Lin *et al* indicates that NFκB activation leads to apoptosis in other cell lines.

The Examiner's attention is also drawn to Ravi *et al*, 2001, *Nature Cell Biology*, 3: 409-415. Ravi *et al* discusses the role of the Rel A and c-Rel subunits of NFκB and indicates that NFκB can both prevent or stimulate apoptosis depending on the cell type and stimulus. As indicated in Ravi *et al* at page 414, left hand column, second paragraph, in reviewing what was known about NFκB:

> *"These observations raise a fundamental issue of how NFκB* can have divergent *effects on cell survival depending on the cell type and the specific activating signal."* [emphasis added]

In discussing possible mechanisms, Ravi *et al* indicates at page 414, right hand column, first full paragraph refers to NFκB subunit dimers states that:

> *"it is also conceivable that dimers composed of either subunit could have different effects depending on the cell type and the circumstances of activation and duration."* [emphasis added]

Thus, at the time of the invention the skilled person would have been well aware of the complexity of NFκB's role in apoptotic pathways. In particular, the skilled person would know that NFκB could both play a role in inducing and inhibiting apoptosis and that results vary from cell type to cell type and on the context of the stimulus.

The skilled person would not therefore have extrapolated from results seen in T cells to HSCs with any reasonable expectation of success and indeed would have considered there a possibility that sulfasalazine would inhibit apoptosis of HSCs or have had no effect. Thus, it would not have been obvious to treat liver fibrosis with sulfasalazine due to any references in Liptay *et al* to NFκB and inducing apoptosis in T cells.

Thus, in summary, the skilled person:

- would not have had a reasonable expectation of success that artificial induction of HSC apoptosis could be used to treat liver fibrosis and in reality would have considered that it could have a detrimental effect; and

- would have considered that sulfasalazine could actually have inhibited HSC apoptosis or had no effect on HSCs in the absence of any indication in the prior art of how sulfasalazine would effects HSCs.

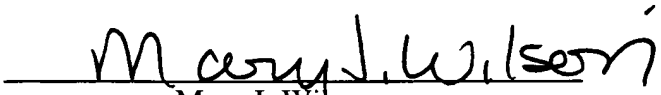
Either factor would individually be enough for the subject matter of the claims to be non-obvious. Taken together, they mean that there can be no question that is the case. Reconsideration is requested.

The Examiner is requested to initial and return the PTO/SB/08a Form submitted herewith.

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: 
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